Current Biology 20, 149–155, January 26, 2010 ©2010 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2009.11.035

Report

A Cellular Memory of Developmental History Generates Phenotypic Diversity in *C. elegans*

Sarah E. Hall,[1,3,*] Matthew Beverly,[1,3] Carsten Russ,[2] Chad Nusbaum,[2] and Piali Sengupta[1,*]

[1]Department of Biology and National Center for Behavioral Genomics, Brandeis University, Waltham, MA 02454, USA

[2]The Broad Institute, Cambridge, MA 02142, USA

Summary

Early life experiences have a major impact on adult phenotypes [1–3]. However, the mechanisms by which animals retain a cellular memory of early experience are not well understood. Here we show that adult wild-type *Caenorhabditis elegans* that transiently pass through the stress-resistant dauer larval stage exhibit distinct gene expression profiles and life history traits, as compared to adult animals that bypassed this stage. Using chromatin immunoprecipitation experiments coupled with massively parallel sequencing, we found that genome-wide levels of specific histone tail modifications are markedly altered in postdauer animals. Mutations in subsets of genes implicated in chromatin remodeling abolish, or alter, the observed changes in gene expression and life history traits in postdauer animals. Modifications to the epigenome as a consequence of early experience may contribute in part to a memory of early experience and generate phenotypic variation in an isogenic population.

Results and Discussion

Early developmental or environmental experience profoundly affects adult behaviors. The effects of modality-specific experience during early "critical periods" for the correct development of defined neural circuits is well studied (reviewed in [4]). Early experiences also have more general effects on animal behavior and development. For instance, childhood abuse has been linked to increased rates of mental and behavioral disorders in adult humans [2, 3]. The molecular mechanisms by which early experience results in global, long-lasting changes in the adult are poorly understood.

Early environmental experience plays a major role in the life cycle of the nematode *Caenorhabditis elegans*. Environmental cues are assessed prior to the first larval molt to regulate the decision between entry into the stress-resistant dauer developmental stage or continuation in the reproductive cycle at the second larval molt (Figure 1A) [5, 6]. When conditions improve, dauer larvae exit the dauer stage and resume reproductive growth. However, despite their starkly distinct developmental histories, phenotypic differences between adults that bypassed the dauer stage (henceforth referred to as control animals) and animals that transiently passed through the dauer stage (henceforth referred to as postdauer animals) (Figure 1A) have previously been uncovered only in mutant backgrounds [7–9], leaving open the question of whether a memory of developmental history also influences phenotypes in wild-type control and postdauer adults.

To address this issue at the organismal level, we first investigated whether control and postdauer wild-type adult animals exhibited global differences in gene expression. To ensure that populations were matched for age and growth conditions, we developed a protocol for precise regulation of dauer entry and exit (see Supplemental Experimental Procedures available online) and compared the expression profiles of control and postdauer animals that had remained in the dauer stage for 1 day. The expression of 1181 and 946 genes was significantly up- or downregulated, respectively, in postdauer animals compared to controls (Tables S1 and S2). Expression changes in postdauer animals ranged from an 8-fold decrease to a 5-fold increase compared to control expression levels; however, ~60% of these genes exhibited expression changes that were altered by less than 1.5-fold (Figure 1B; Tables S1 and S2). Because the expression profiles of whole adult animals were examined, we expect that these smaller expression changes are biologically relevant and may result from an overall small change in expression in many tissues or large changes in a subset of expressing cells. Expression changes predicted from microarray hybridization experiments were further verified via quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) (Figure S1A).

In order to determine whether specific biological processes were preferentially affected upon passage through the dauer stage, we identified the Gene Ontology (GO) terms [10] associated with each gene exhibiting altered expression in postdauer animals. Affected genes were associated with multiple cellular and biological pathways; overrepresented categories included genes predicted to encode proteins involved in regulation of the cell cycle, transcription, phosphorylation and dephosphorylation, metabolism, reproduction, and G protein-coupled receptor signaling (Table S3). Expression changes of subsets of these genes in different categories are summarized in Table S2.

Because one of the largest groups of genes identified was associated with reproduction, we further compared this data set with genes previously identified as sperm- or oocyte-enriched in expression profiling experiments [11]. The expression of 23% of genes identified as sperm-enriched was significantly downregulated in postdauer animals, whereas the expression of only 1% was upregulated (Figure 1B). Notably, the expression of 65 of 88 predicted major sperm cytoskeletal protein (*msp*) genes implicated in oocyte maturation, sheath cell contraction, ovulation, and sperm movement [12, 13] was downregulated in postdauer animals (Table S1). In contrast, the expression of 32% of oocyte-enriched genes was upregulated in postdauer animals, and none were downregulated (Figure 1B; Table S1). Passage through the dauer stage has been shown to increase male survival and facultative outcrossing rates in some predominantly self-fertilizing *C. elegans* isolates [14]. Together, these results suggest that changes in gene expression may contribute to altered life history traits such as reproduction in postdauer adults.

To determine when altered gene expression profiles arise in postdauer adults, we examined expression levels of the set of

*Correspondence: sehall@brandeis.edu (S.E.H.), sengupta@brandeis.edu (P.S.)

[3]These authors contributed equally to this work

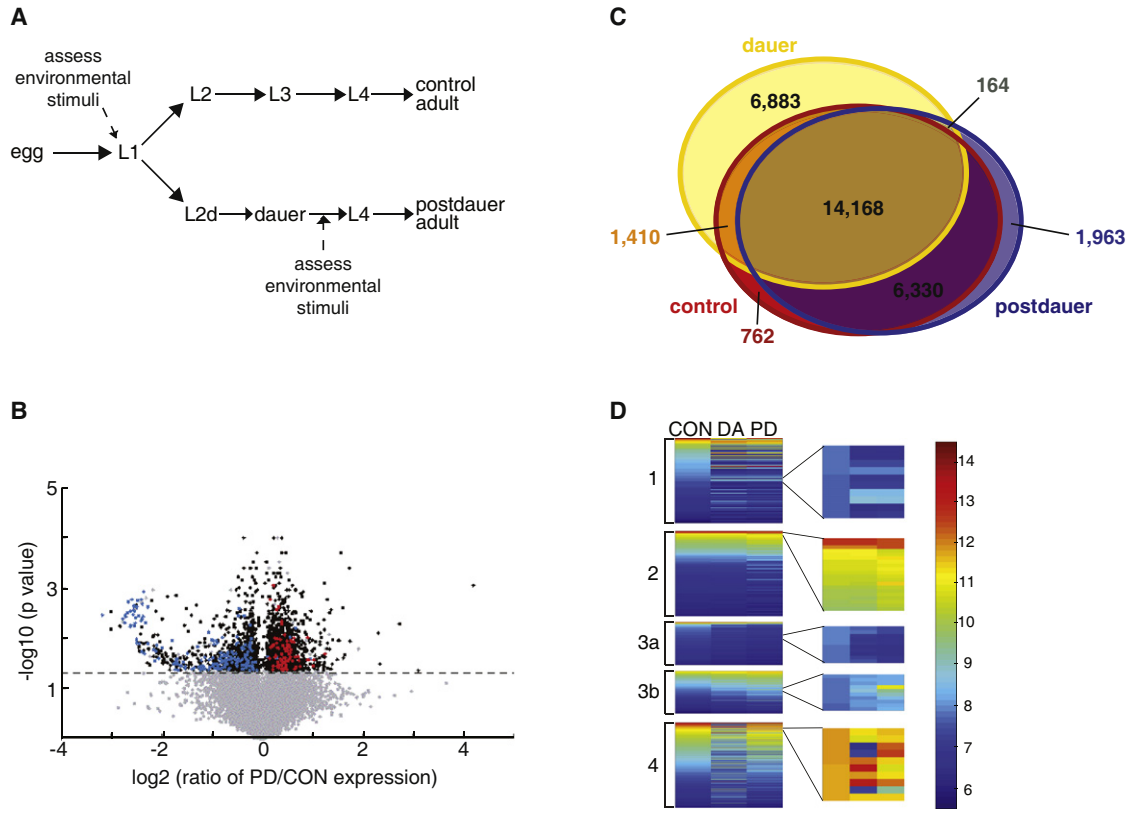

Figure 1. Control and Postdauer Adult Animals Exhibit Distinct Gene Expression Profiles

(A) *C. elegans* may develop via one of two alternative pathways, resulting in adults with distinct developmental histories.

(B) Volcano plot showing gene expression differences between populations of control and postdauer adult animals. Data shown are an average from three independent biological replicates. Values on the x axis indicate the log2-transformed values of the fold change in expression; values on the y axis indicate the −log10-transformed p values generated from a two-sample t test with a false discovery rate of <0.25. The horizontal dashed line represents the threshold p value of 0.05. Genes identified as sperm or oocyte enriched are indicated in blue and red, respectively. The following abbreviations are used: CON, control; PD, postdauer.

(C) Venn diagram of all probes represented on the microarray showing comparison of expression levels among controls, dauers that were in the dauer stage for 1 day, and postdauer adult animals. The subset of genes (represented by 1963 + 164 probes) whose expression was significantly different in postdauer adults as compared to control adults was further categorized based on expression in (D).

(D) Heat maps of gene expression changes in control (CON), dauer (DA), and postdauer (PD) animals. Blow-ups of selected regions are shown at right. Genes were sorted into four groups based on expression changes and were further sorted based on expression levels in control animals. Genes in group 3 were further subdivided based on the direction of gene expression change between dauer and postdauer animals relative to expression in control animals (3a, downregulated; 3b, upregulated). Eleven probes in group 1 show large variance in expression levels between replicates, resulting in apparent expression level differences between dauer and postdauer samples. Gene Ontology (GO) functional terms for genes in each group are shown in Table S3. Color key indicates normalized log2-transformed expression values. See also Figure S1.

2127 genes described above in dauer animals that were in the dauer stage for 1 day (Figure 1C). Based on expression in control, dauer, and postdauer animals, these genes were further clustered into four groups (Figure 1D; Table S1). Genes in group 1 (164 genes) exhibited similar expression levels in postdauer adults and dauer animals, indicating that the expression levels of these genes were altered during the dauer stage and stably maintained thereafter (Figure 1D). The expression levels of genes in group 2 (143 genes) were similar in dauer animals and control adults but were altered (either up- or downregulated) in postdauer animals (Figure 1D), indicating that altered expression of this gene set in postdauer adults was likely to occur following exit from the dauer stage. Group 3 (400 genes) included genes whose expression levels were either up- or downregulated in both dauer and postdauer animals, compared to controls (Figure 1D). Expression of members of this group was altered in the dauer stage, and was further up- or downregulated in postdauer adults. The largest group (group 4; 1420 genes) included genes whose expression levels were distinct and uncorrelated in control, dauer, and postdauer adults (Figure 1D). These results indicate that the altered gene expression observed in postdauer animals arises from multiple regulatory mechanisms acting both during the dauer stage as well as upon subsequent resumption of reproductive growth. Moreover, because genes associated with specific GO functional terms were overrepresented in different groups (Table S3), genes with related functions may be regulated via similar mechanisms. Previous work identified genes whose expression is altered over a 12 hr time course during the transition from the dauer to the postdauer stage [15]. However, there was little overlap of genes identified in this work with either the dauer or the postdauer gene sets described here (Figure S1B), likely as a result of differences in experimental methods ([15]; see Supplemental Experimental Procedures).

The expression of chromatin-associated genes such as histones was previously shown to be altered in the dauer stage [16], and we identified genes implicated in nucleosome

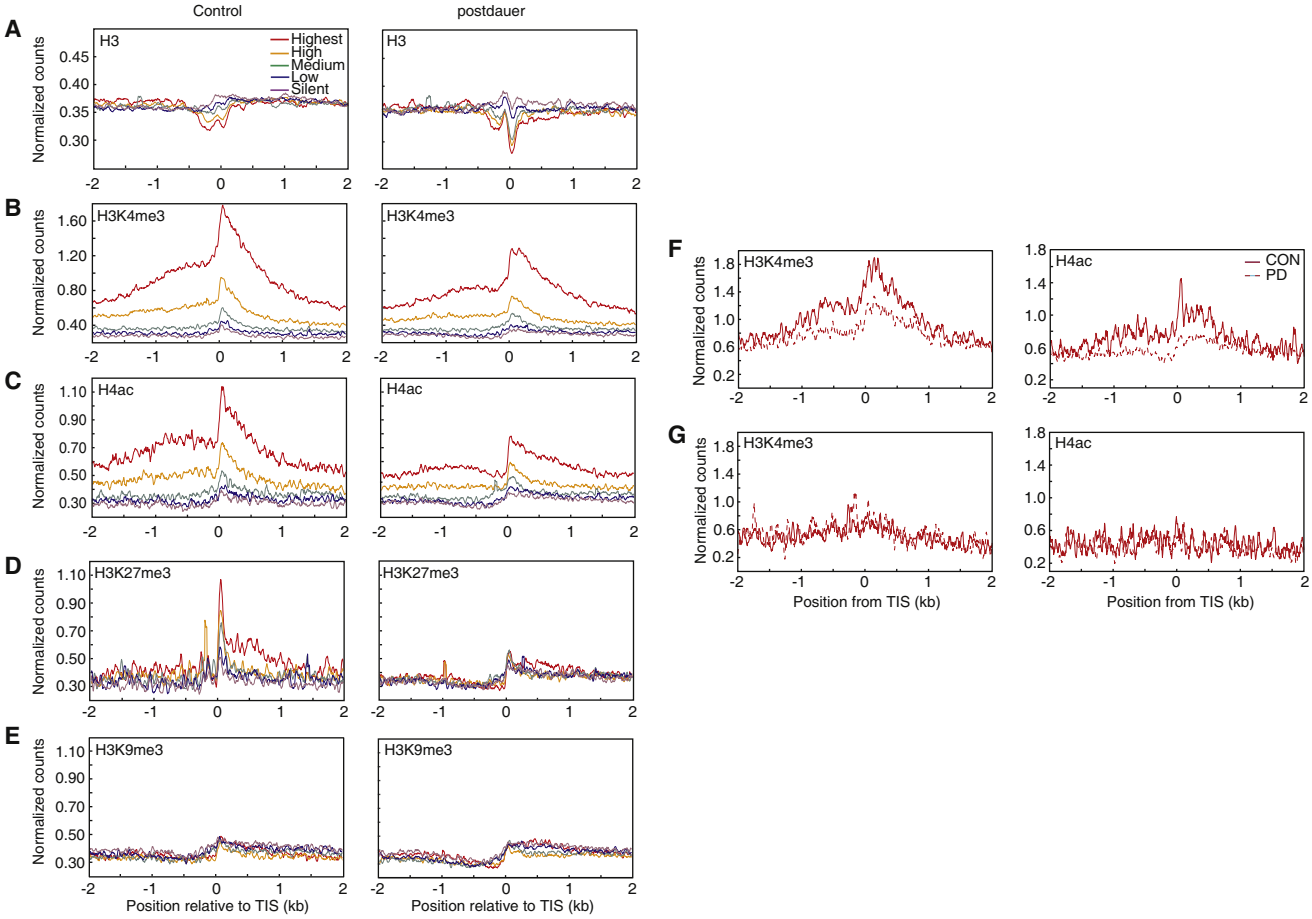

Figure 2. The Genome-wide Chromatin State of Postdauer Animals Is Distinct from that in Control Animals

(A–E) The average number of normalized sequence reads were obtained from ChIP-Seq experiments with antibodies against histone H3 (A), H3K4me3 (B), H4ac (C), H3K27me3 (D), and H3K9me3 (E) for five groups of ~4000 genes each, categorized based on expression levels in control or postdauer animals. Positions relative to the translation initiation sites (at 0) for each gene are indicated on the x axis.

(F and G) The average number of normalized sequence reads in control and postdauer adults with antibodies against H3K4me3 and H4ac for the highest expressed gene group (~200 genes) in the upregulated (F) or downregulated (G) gene sets identified by transcriptional profiling. Quantification and statistical analyses are shown in Figure S2 and Table S4. Antibodies used and the numbers of reads for each experiment are indicated in Table S5. The overall ratio of histone H3 levels of postdauer animals compared to control animals was confirmed to be similar by western blot ($H3_{PD}/H3_{CON}$ = 1.07).

assembly and chromatin remodeling in the gene set whose expression is altered in postdauer animals (Tables S1 and S2). We investigated whether the observed developmental experience-dependent changes in gene expression profiles were associated with global alterations in the epigenome. We performed ChIP-Seq (chromatin immunoprecipitation followed by sequencing) [17, 18] analyses to generate genome-wide maps of histone modifications with DNA isolated from populations of control and postdauer animals grown under conditions similar to those used for transcriptional profiling. ChIP was performed with antibodies directed against histone H3 to assess nucleosome density as well as against histone modifications associated with euchromatin (pan-acetylation of histone H4 [H4ac] and trimethylation of histone H3 at lysine 4 [H3K4me3]) and heterochromatin (trimethylation of histone H3 at lysine 9 [H3K9me3] and lysine 27 [H3K27me3]).

We first correlated histone modifications in gene regulatory and coding sequences with overall expression levels in control and postdauer animals. Because few gene transcriptional start sites are known in *C. elegans* as a result of *trans*-splicing [19], we instead averaged the number of ChIP-Seq reads for each histone modification in the regions 2 kb up- and downstream of the translation initiation sites (TISs) and compared the histone modification profiles with gene expression levels. Nucleosome occupancy and overall histone H3 levels were similar in control and postdauer animals regardless of gene expression levels (Figure 2A), suggesting that histone modifications, rather than nucleosome content, may be correlated with gene activity under the examined conditions.

Unexpectedly, we found that H3K4me3 and H4ac modifications were decreased genome-wide in postdauer animals, despite similar overall gene expression levels (Figures 2B and 2C; Figures S2A and S2B; Table S4). This decrease was primarily observed for highly expressed genes and not genes expressed at lower levels, perhaps because of a floor effect in detection sensitivity. H3K27me3 and H3K9me3 levels were similar in both control and postdauer animals (Figures 2D and 2E; Table S4). H3K4me3 and H4ac modifications were positively correlated with gene expression levels across gene regulatory and coding sequences in both control and postdauer animals (Figures 2B and 2C; Figures S2A and S2B; Table S4) [20]. In contrast, H3K27me3 and H3K9me3

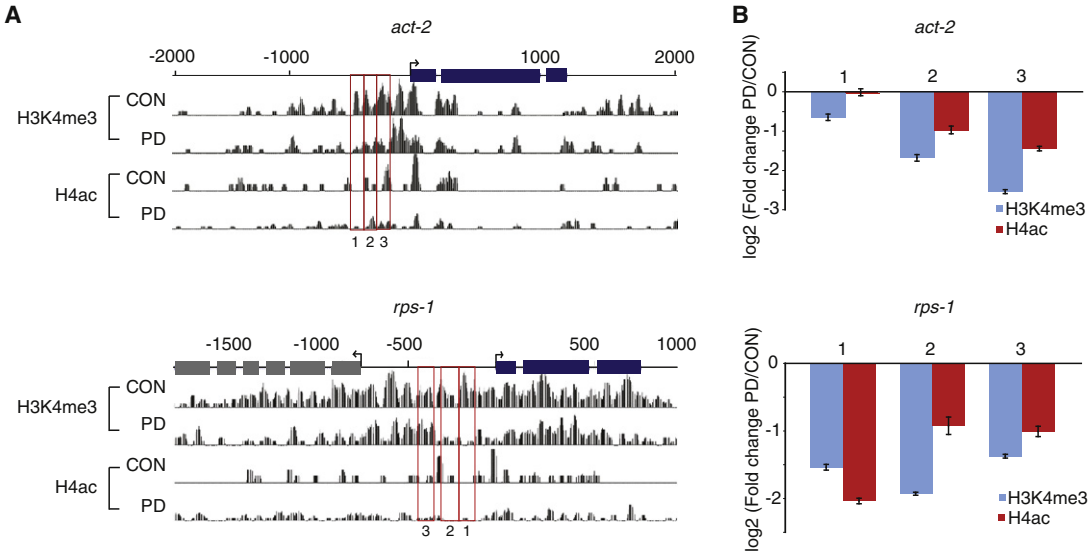

Figure 3. H3K4me3 and H4ac Levels Are Decreased at Individual Loci in Postdauer Animals

(A) Histone modification profiles in the regulatory and transcribed regions of the *act-2* actin and *rps-1* ribosomal protein subunit genes in control (CON) and postdauer (PD) animals displayed as custom tracks on the UCSC genome browser. Exons of these two genes are indicated by blue boxes.

(B) Log2-transformed ratios of postdauer to control levels of the indicated histone modifications across three 100 bp sequences in the regulatory regions (indicated by red boxes with corresponding numbers in A) of *act-2* and *rps-1*. Chromatin immunoprecipitation followed by quantitative polymerase chain reaction was performed on a biologically independent population of control and postdauer animals. Error bars indicate propagated standard deviations of technical replicates.

modifications were not strongly correlated with gene expression levels (Figures 2D and 2E; Figures S2A and S2B; Table S4), as has previously been observed in human CD4+ T cells [17]. However, H3K9me3 levels were previously shown to be enriched across genes expressed at low levels in *C. elegans* [20]. Differences in the developmental stages of animals, or the methodologies used in the two studies, may account in part for these different observations.

To further examine the observed changes in chromatin state in postdauer animals, we selected individual genes from the highest expressed category and quantified levels of H3K4me3 and H4ac modifications across their regulatory and coding sequences. Average levels of both modifications were decreased across both upstream and downstream sequences of examined genes (Figure 3A), although their overall expression levels were unaltered. To verify the chromatin modification level changes, we performed chromatin immunoprecipitation from a biologically independent population of control and postdauer adult animals and quantified histone modification levels across the regulatory sequences by quantitative PCR. We again observed decreases in both HeK4me3 and H4ac levels in the sequences examined (Figure 3B). Taken together, these results imply that the genome-wide chromatin modification profiles in postdauer animals are markedly distinct from those in control animals.

Because the expression of only ~10% of predicted genes was altered in postdauer animals (Figure 1B), any chromatin changes specific to this subset may be masked in the genome-wide histone modification maps. We therefore next examined the chromatin states associated specifically with this gene set in control and postdauer animals. The 2127 genes identified via transcriptional profiling were first separated into two groups based on whether expression was up- or downregulated in postdauer animals and were further binned based on expression levels. We found that the overall chromatin state changes were similar to those observed in the genome-wide experiments, such that global levels of both H3K4me3 and H4ac modifications were decreased in the highest expressed gene category in postdauer animals (Figure 2F; Figures S2C–S2F; Table S4). This decrease was primarily observed for the upregulated but not the downregulated genes, presumably because of overall lower levels of these modifications in the downregulated gene set (Figures 2F and 2G). Levels of both modifications were positively correlated with gene activity in both the up- and downregulated gene subsets (Figures S2C–S2F; Table S4). No correlation was observed between the fold change in expression and the chromatin modification profiles when comparing control and postdauer populations (Figures S2G–S2J), although this analysis does not preclude correlations at the level of individual genes. These results indicate that the gene subset whose expression is altered in postdauer animals is subject to overall developmental history-dependent chromatin modification changes similar to those observed across the genome. Changes in the expression of individual genes may, therefore, arise from additional genetic or epigenetic mechanisms acting at the local level.

To begin to identify mechanisms that may play a role in the regulation of gene expression and reproduction in postdauer animals, we next investigated whether mutations in chromatin remodeling genes affect postdauer expression changes. We surveyed postdauer gene expression changes of the highly downregulated major sperm protein gene *msp-64* and the highly upregulated choline/carnitine O-acyltransferase gene *W03F9.4* in mutants defective in different chromatin remodeling pathways (Figures 4A and 4B). The selected mutants were viable and did not exhibit defects in entry into, or exit from, the dauer stage. Mutations in many of the selected chromatin remodeling genes affected expression levels in control adult animals (Figures S3A and S3B); we therefore determined whether the ratio of postdauer to control expression was

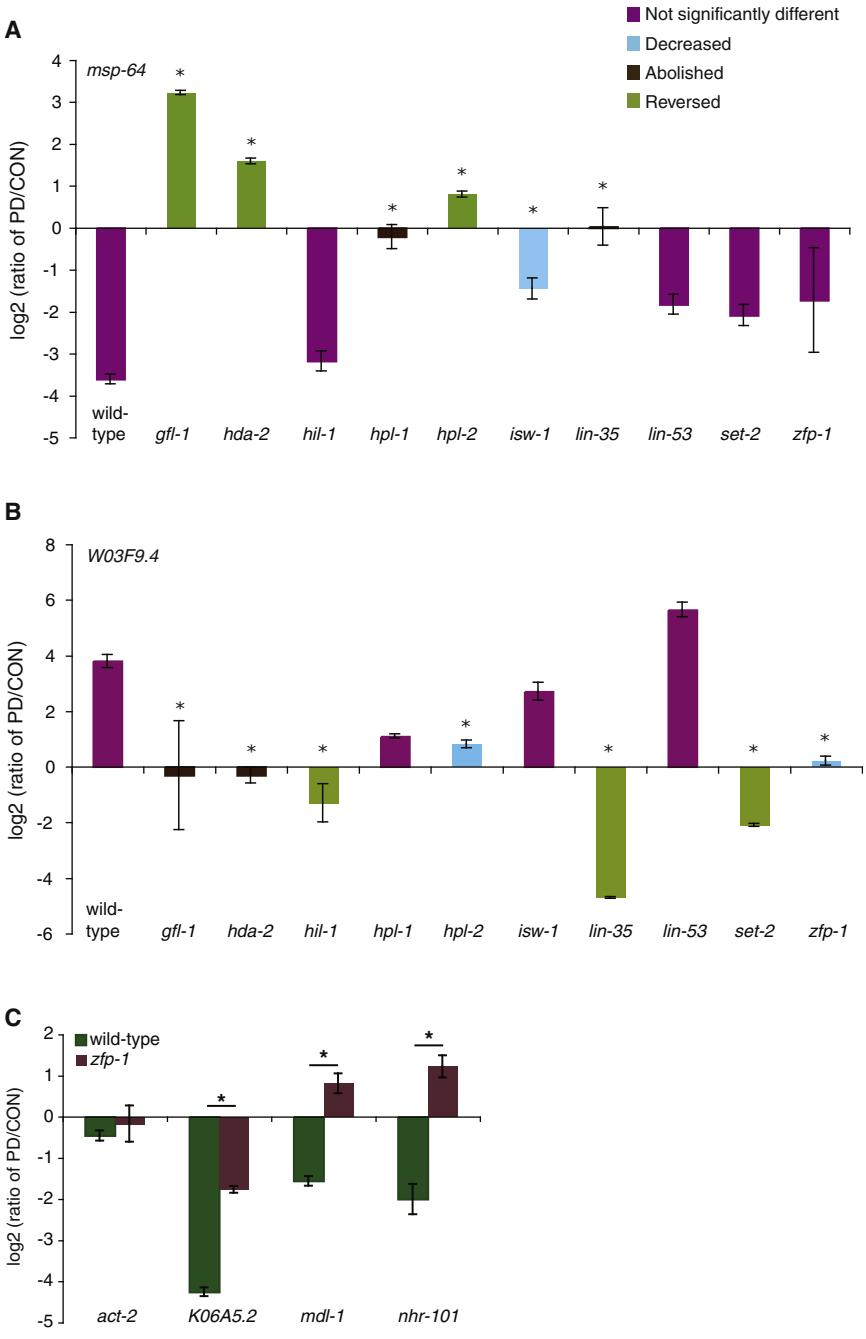

Figure 4. Mutations in Genes Implicated in Chromatin Remodeling Affect Postdauer Gene Expression Changes

(A and B) Expression ratio of *msp-64* (A) and *W03F9.4* (B) between postdauer and control animals (log2-transformed) in the indicated genetic backgrounds. Gene expression was examined in animals mutant for the *zfp-1* and *gfl-1* chromatin-associated proteins, *hda-2* histone deacetylase, *hpl-1* and *hpl-2* HP-1-like proteins, *isw-1* chromatin remodeling ATPase, *lin-35* retinoblastoma tumor suppressor ortholog, *lin-53* nucleosome remodeling complex member, *hil-1* H1-like histone, and the *set-2* histone H3 (K4) methyltransferase complex member genes. *hil-1* levels have been shown to be upregulated in dauer animals [16], and we found that the spatial pattern, but not overall expression levels of *hil-1*, was altered in postdauer animals (Figures S3D and S3E). See Supplemental Experimental Procedures for alleles used. Error bars represent standard error of the mean (SEM). $n \geq 6$; ≥ 2 independent assays. Asterisks indicate values that are different from those in wild-type animals at $p < 0.05$. See also Figure S3.

(C) Expression ratio of (log2-transformed) indicated genes in postdauer and control wild-type and *zfp-1* mutant animals. Error bars represent SEM. $n \geq 6$; ≥ 2 independent assays. Asterisks indicate values that are different from those in wild-type animals at $p < 0.05$.

significantly different between wild-type and mutant animals. We found that although the expression changes of *msp-64* and *W03F9.4* in a subset of mutant postdauer animals were similar to those in wild-type postdauer animals, mutations in additional chromatin remodeling genes either decreased, abolished, or reversed the expected levels of up- or downregulation in postdauer animals (Figures 4A and 4B). Each mutation had different effects on *msp-64* and *W03F9.4* expression. Similarly, the postdauer expression changes of three additional genes identified by transcriptional profiling were differentially affected in animals mutant for the *zfp-1* chromatin-associated zinc finger protein gene [21, 22], although the postdauer expression of the *act-2* actin gene remained unaltered (Figure 4C; Figure S3C). These observations imply that remodeling of chromatin architecture plays a causal role in the establishment or maintenance of the postdauer expression changes and that different mechanisms may affect the expression of different gene sets.

We next determined whether the altered gene expression and chromatin modification profiles were associated with altered phenotypes in postdauer adult animals. Because the expression of a number of genes implicated in the regulation of *C. elegans* adult life span and reproduction was affected in postdauer animals (Tables S1–S3), we quantified the adult life span and brood sizes of postdauer and control animals. We found that the mean adult life span of postdauer animals was significantly extended when compared to values in control animals (Figure 5A). Additionally, postdauer animals produced more progeny than controls (Figure 5B). Discrepancies between these and previous observations [23, 24] are likely due to comparison of non-age-matched populations of control and postdauer animals or induction of dauer entry through the use of nonphysiological methods in previous reports [24, 25]. Similar to their effects on gene expression changes in postdauer animals, mutations in a subset of chromatin remodeling genes abolished the brood size differences between control and postdauer animals (Figure 5B). The embryonic lethality and adult survival rates were similar between the control and postdauer populations in all genetic backgrounds (Figures S4A and S4B). These results indicate that the developmental experience of spending one day in

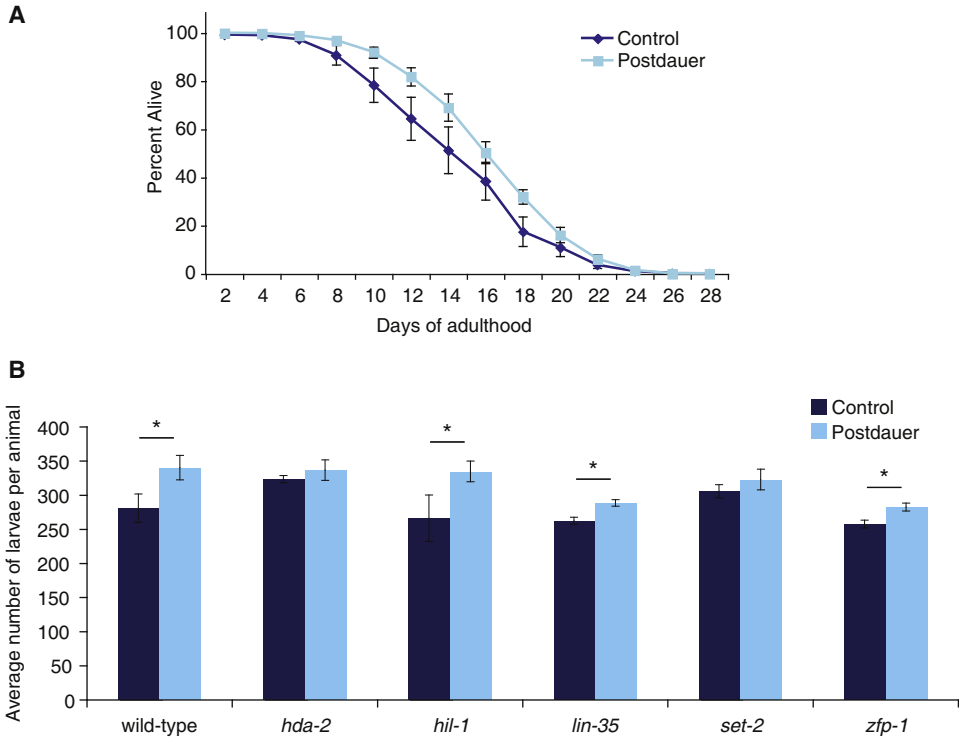

Figure 5. Postdauer Animals Exhibit a Longer Mean Life Span and Have a Larger Brood Size than Control Animals

(A) Survival curves of control and postdauer animals. Day 0 corresponds to the L4 larval stage. Mean life span of control and postdauer adult animals was 12.9 ± 0.2 and 14.8 ± 0.2 days, respectively ($p < 0.05$; Student's t test). Life span curves include censored data (see Supplemental Experimental Procedures) ($p < 0.0001$; log rank [Wilcoxon-Gehan] test). Maximum life span of control and postdauer animals was 21.0 ± 1.5 and 21.6 ± 1.4 days, respectively. Error bars represent SEM. $n > 500$ for each; 4 independent assays.

(B) The average number of larvae produced by control and postdauer animals of the indicated genetic backgrounds. See Supplemental Experimental Procedures for alleles used. Error bars represent SEM. Asterisks indicate values that are significantly different at $p < 0.05$ between the indicated conditions. $n > 24$ animals each. See also Figure S4.

the dauer stage is sufficient to significantly alter physiology and life history traits in postdauer animals and further support the notion that changes in chromatin state may be causal to these phenotypic differences.

Brood sizes in the self-fertilizing *C. elegans* hermaphrodite are limited by the number of sperm produced; animals lay unfertilized oocytes once sperm are depleted [26]. To determine whether increased spermatogenesis may account for the increased brood size in postdauer animals, we quantified the time course of progeny production by control and postdauer animals. Although the rate of egg laying was unaltered between the two populations (Figure S4C), postdauer animals produced more progeny on later days, suggesting that total sperm number may be increased in postdauer animals (Figure S4D). Moreover, sperm from control males was similarly effective in competing with endogenous sperm for the production of progeny in both control and postdauer animals (Figure S4E). These results indicate that under the examined conditions, the observed downregulation of sperm-enriched genes in postdauer animals (Figure 1B) may be compensated for by other mechanisms, resulting in a larger brood size.

Conclusions

Our results indicate that *C. elegans* retains a cellular memory of its developmental history that is reflected in altered life history traits, gene expression, and global chromatin state in postdauer animals. These experience-dependent changes in chromatin modifications may serve as a global signature of the specific developmental experience of transient passage through the dauer stage. The chromatin state of dauer animals has also been proposed to be distinct [16] and may contribute to the observed changes in postdauer animals. At a subset of loci, additional chromatin modifications or other transcriptional mechanisms may act at the local level to establish or maintain expression changes in postdauer animals. The complexity of these regulatory mechanisms is reflected in the different temporal patterns of gene expression changes in postdauer animals, as well as the differential effects of chromatin remodeling genes on the expression changes of individual genes. We suggest that at other loci, these global modifications poise genes for further regulation by additional mechanisms upon subsequent exposure to specific external or internal cues. This is analogous to key regulatory genes in embryonic stem cells being associated with "bivalent" marks of H3K4 and H3K27 methylation, allowing either gene activation or silencing upon lineage-specific differentiation [18, 27].

Alteration in gene expression, and consequent phenotypic differences among animals exposed to different environmental or development experiences, creates phenotypic variation in a genetically identical population and may provide critical evolutionary advantages. In rodents and humans, early experience shapes adult behaviors via alterations in DNA methylation state and histone modification profiles at specific loci [28–30]. Similarly, early mechanosensory stimulation has

been shown to modulate adult behavior and gene expression in *C. elegans* [31]. The establishment of *C. elegans* as a model system in which to explore the roles of early experience on adult phenotypes now allows for studies of the underlying genetic and epigenetic mechanisms at high resolution in an experimentally tractable organism.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, four figures, and five tables and can be found with this article online at doi:10.1016/j.cub.2009.11.035.

Acknowledgments

We are grateful to A. Grishok, B. Bernstein, P. Kolasinska-Zwierz, and S. Young for advice and technical assistance and the Caenorhabditis Genetics Center for providing strains. We thank A. Grishok, M. Marr, O. Hobert, C. Bargmann, and J. Haber for comments on the manuscript and the Sengupta laboratory for discussion. This work was supported by the National Institutes of Health (RO1 GM56223 to P.S. and F32 GM083593 to S.E.H.) and by a National Science Foundation Integrative Graduate Education and Research Traineeship training grant (DGE-0549390 to M.B.).

Received: July 13, 2009
Revised: October 13, 2009
Accepted: November 9, 2009
Published online: January 14, 2010

References

1. Youngentob, S.L., and Glendinning, J.I. (2009). Fetal ethanol exposure increases ethanol intake by making it smell and taste better. Proc. Natl. Acad. Sci. USA *106*, 5359–5364.
2. Graham, Y.P., Heim, C., Goodman, S.H., Miller, A.H., and Nemeroff, C.B. (1999). The effects of neonatal stress on brain development: Implications for psychopathology. Dev. Psychopathol. *11*, 545–565.
3. Heim, C., and Nemeroff, C.B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: Preclinical and clinical studies. Biol. Psychiatry *49*, 1023–1039.
4. Hensch, T.K. (2004). Critical period regulation. Annu. Rev. Neurosci. *27*, 549–579.
5. Golden, J.W., and Riddle, D.L. (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science *218*, 578–580.
6. Golden, J.W., and Riddle, D.L. (1984). The *Caenorhabditis elegans* dauer larva: Developmental effects of pheromone, food, and temperature. Dev. Biol. *102*, 368–378.
7. Euling, S., and Ambros, V. (1996). Reversal of cell fate determination in *Caenorhabditis elegans* vulval development. Development *122*, 2507–2515.
8. Cox, G.N., Laufer, J.S., Kusch, M., and Edgar, R.S. (1980). Genetic and phenotypic characteristics of roller mutants of *Caenorhabditis elegans*. Genetics *95*, 317–339.
9. Sutherlin, M.E., and Emmons, S.W. (1994). Selective lineage specification by *mab-19* during *Caenorhabditis elegans* male peripheral sense organ development. Genetics *138*, 675–688.
10. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., et al. The Gene Ontology Consortium. (2000). Gene ontology: Tool for the unification of biology. Nat. Genet. *25*, 25–29.
11. Reinke, V., Smith, H.E., Nance, J., Wang, J., Van Doren, C., Begley, R., Jones, S.J., Davis, E.B., Scherer, S., Ward, S., and Kim, S.K. (2000). A global profile of germline gene expression in *C. elegans*. Mol. Cell *6*, 605–616.
12. Ward, S., Burke, D.J., Sulston, J.E., Coulson, A.R., Albertson, D.G., Ammons, D., Klass, M., and Hogan, E. (1988). Genomic organization of major sperm protein genes and pseudogenes in the nematode *Caenorhabditis elegans*. J. Mol. Biol. *199*, 1–13.
13. Miller, M.A., Nguyen, V.Q., Lee, M.H., Kosinski, M., Schedl, T., Caprioli, R.M., and Greenstein, D. (2001). A sperm cytoskeletal protein that signals oocyte meiotic maturation and ovulation. Science *291*, 2144–2147.
14. Morran, L.T., Cappy, B.J., Anderson, J.L., and Phillips, P.C. (2009). Sexual partners for the stressed: Facultative outcrossing in the self-fertilizing nematode Caenorhabditis elegans. Evolution *63*, 1473–1482.
15. Wang, J., and Kim, S.K. (2003). Global analysis of dauer gene expression in *Caenorhabditis elegans*. Development *130*, 1621–1634.
16. Jones, S.J., Riddle, D.L., Pouzyrev, A.T., Velculescu, V.E., Hillier, L., Eddy, S.R., Stricklin, S.L., Baillie, D.L., Waterston, R., and Marra, M.A. (2001). Changes in gene expression associated with developmental arrest and longevity in *Caenorhabditis elegans*. Genome Res. *11*, 1346–1352.
17. Barski, A., Cuddapah, S., Cui, K., Roh, T.Y., Schones, D.E., Wang, Z., Wei, G., Chepelev, I., and Zhao, K. (2007). High-resolution profiling of histone methylations in the human genome. Cell *129*, 823–837.
18. Mikkelsen, T.S., Ku, M., Jaffe, D.B., Issac, B., Lieberman, E., Giannoukos, G., Alvarez, P., Brockman, W., Kim, T.K., Koche, R.P., et al. (2007). Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature *448*, 553–560.
19. Blumenthal, T. (1995). Trans-splicing and polycistronic transcription in *Caenorhabditis elegans*. Trends Genet. *11*, 132–136.
20. Kolasinska-Zwierz, P., Down, T., Latorre, I., Liu, T., Liu, X.S., and Ahringer, J. (2009). Differential chromatin marking of introns and expressed exons by H3K36me3. Nat. Genet. *41*, 376–381.
21. Kim, J.K., Gabel, H.W., Kamath, R.S., Tewari, M., Pasquinelli, A., Rual, J.F., Kennedy, S., Dybbs, M., Bertin, N., Kaplan, J.M., et al. (2005). Functional genomic analysis of RNA interference in *C. elegans*. Science *308*, 1164–1167.
22. Dudley, N.R., Labbé, J.C., and Goldstein, B. (2002). Using RNA interference to identify genes required for RNA interference. Proc. Natl. Acad. Sci. USA *99*, 4191–4196.
23. Klass, M., and Hirsh, D. (1976). Non-ageing developmental variant of *Caenorhabditis elegans*. Nature *260*, 523–525.
24. Kim, S., and Paik, Y.K. (2008). Developmental and reproductive consequences of prolonged non-aging dauer in *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. *368*, 588–592.
25. Gallo, M., and Riddle, D.L. (2009). Effects of a *Caenorhabditis elegans* dauer pheromone ascaroside on physiology and signal transduction pathways. J. Chem. Ecol. *35*, 272–279.
26. Ward, S., and Carrel, J.S. (1979). Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. Dev. Biol. *73*, 304–321.
27. Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell *125*, 315–326.
28. Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., and Meaney, M.J. (2004). Epigenetic programming by maternal behavior. Nat. Neurosci. *7*, 847–854.
29. Meaney, M.J., Diorio, J., Francis, D., Weaver, S., Yau, J., Chapman, K., and Seckl, J.R. (2000). Postnatal handling increases the expression of cAMP-inducible transcription factors in the rat hippocampus: The effects of thyroid hormones and serotonin. J. Neurosci. *20*, 3926–3935.
30. McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., Labonté, B., Szyf, M., Turecki, G., and Meaney, M.J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat. Neurosci. *12*, 342–348.
31. Ebrahimi, C.M., and Rankin, C.H. (2007). Early patterned stimulation leads to changes in adult behavior and gene expression in *C. elegans*. Genes Brain Behav. *6*, 517–528.